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Phenotypic Variations and Switches in HIV Isolated From the Blood and the Gastrointestinal Tissues of Patients With HIV-1 Infection

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The objective of this study was to determine the initial and subsequent phenotypes of HIV-1 isolated from the blood, duodenal, and colonic biopsies of 51 HIV-1 positive patients followed prospectively over 2 years. Blood and tissues were cocultured with stimulated peripheral blood monocytes, and HIV was analyzed for phenotypic expression of syncytia-induction (SI). A total of 45/51 patients had HIV-1 isolated from the blood and 35/51 had HIV isolated from gastrointestinal tract. In 12/45 patients SI-HIV-1 was isolated from the blood. In 6/12 patients the blood phenotype reverted to the NSI phenotype. SI phenotypes were also isolated from the colon and duodenum of 8/35 patients and reversion from SI to NSI virus phenotype was again observed in gut tissue of 3/8 patients. These results show that gastrointestinal tissues can harbor SI HIV phenotype. Discordant phenotypes can be found in tissue and blood of late-stage patients. Reversion of phenotypic SI expression to NSI may occur in patients receiving monotherapy as antiretroviral treatment. These results suggest that gastrointestinal tissues may act as a separate and distinct site involved in HIV replication and its associated pathogenesis. ***J. Med. Virol.* 52:31–34, 1997.** © 1997 Wiley-Liss, Inc.

KEY WORDS: syncytium-inducing (SI) variant; HIV enteropathy; gastrointestinal biopsies; reversion

INTRODUCTION

A heterogeneous population of HIV-1 phenotypes can be detected in a given individual with HIV infection [Tersmette et al., 1988]. Some of these viral variants are able to induce the production of multinucleated giant cells or syncytia in CD4 expressing lymphoblastoid cell lines. These HIV-1 variants usually appear during the later stages of the infection and are often associated with accelerated disease progression [Koot et al., 1993]. In contrast, non-syncytium-inducing (NSI) variants of HIV are usually recovered from patients in the early, asymptomatic stage of the illness, or in patients who have remained well despite being infected by HIV-1 for long periods of time [Koot et al., 1993].

Recovery of syncytium inducing (SI) variants of HIV-1 has so far been documented from peripheral, circulating lymphocytes [Koot et al., 1993; Bozzette et al., 1993] and from the brain biopsies of HIV-1 patients with central nervous system disease [Koyanagi et al., 1987]. HIV-1 infection of the gut has been also documented by isolation of HIV-1 from several sites in the gastrointestinal tract throughout the course of infection [Gelb and Miller, 1987; Ullrich et al., 1989; Gill et al., 1992]. Molecular methods suggest localization of HIV-1 to gut neuroendocrine target cells [Nelson et al., 1988]. Since the gastrointestinal tract is a major lymphoid target of HIV-1 infection, it was important both to determine the type of HIV-1 phenotypic variant recovered from patients in different stages of the illness and to determine if the blood and the gastrointestinal sites harbour similar phenotypes and whether these markers might predict gastrointestinal dysfunction.

Reversion of the SI phenotype to the NSI phenotype appears to be an unusual and rare phenomenon. Karlson and co-workers [1994] have observed a fluctuating phenotype in only one patient out of 53 studied. Delforge et al. [1995] reported the disappearance of the SI phenotype and the appearance of the NSI in the peripheral blood lymphocytes (PBL) of 3 out of 10 HIV-1 positive patients who were undergoing didanosine treatment. They also observed that this reversion was transient and did not occur in patients undergoing other or no anti-retroviral treatment. Zheng and coworkers [1996] also reported a reversion from SI to NSI

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phenotype in 14 patients who underwent DDI monotherapy for 12 weeks. In this study we looked for changes in phenotype expression from the blood and the gastrointestinal tissues of patients throughout various stages of disease progression.

METHODS

Study Group

A total of 51 cases (48 homosexual/bisexual men, 3 women) were enrolled as part of an ongoing comprehensive prospective study of HIV enteropathy and wasting. Cases were sequentially recruited from those attending a regional HIV clinic in 1993 and were followed every 4 months over a minimum of 34 months. Five patients died in the first 2 years since the study began, and 2 withdrew from the study after 2 visits.

Gastrointestinal Biopsies

At every second study visit (i.e., approximately every 8 months), volunteers underwent endoscopy for harvesting biopsies from the duodenum and colon. All gut biopsies are immediately placed into viral transport medium and transported to the retrovirology laboratory within 1 hour of collection.

HIV Tissue Culture and Phenotype Determination

HIV-1 from both peripheral blood lymphocytes (PBL) and gut biopsy tissues was co-cultured with stimulated peripheral blood mononuclear cells from a negative donor using standard methods as previously described (ACTG Virology Manual). Supernatants from positive P24 (i.e., above 0.03 ng/ml) cocultured peripheral blood lymphocytes and/or gut tissue site(s) of individual patients were added onto MT-4 lymphoblastoid cell cultures in RPMI 1640 medium containing 10% fetal calf serum. Negative and positive HIV-1 RF strain control cultures were included in each plate. Cytopathological effects were recorded every 3 days for a total of 2 weeks using standard methodology (ACTG Virology Manual). MT-4 cell cultures were used instead of MT-2 cell lines because they gave a better visual appearance of the syncytium formation.

Viral Load Measurement

HIV-1 plasma RNA quantification was done using the Amplicor HIV monitor test (Roche Diagnostic System, Branchburg, NJ) according to manufacturer's instructions. Briefly, HIV-1 RNA, extracted from 200 μl of cryopreserved plasma samples (–70°C), was added to RNA from an internal quantification standard (QS) which has the same length and priming regions as the HIV target. The extracted RNA was then amplified in a thermal cycler with the use of biotinylated primers SK462 and SK431. Five-fold dilutions of the amplified product were prepared and hybridized in microwell plates containing the probe for HIV and QS. After hybridization, the biotinylated amplicons were reacted with horseradish peroxidase (HRP) conjugate. The substrate was then added and the optical densities were measured at 405 nm. The absorbance results for each specimen were calculated in reference to its own QS.

TABLE I. SI Phenotype Expression Profile of Isolates From the Blood and Gastrointestinal Tissue Biopsies of Selected Patients

Patient no.	HIV-1 variants found in			CD4 count cells/mm^3
	Blood	Colon	Duodenum	
1	SI[a]	NSI	NSI	19
2	SI[a]	NSI	NSI	63
3	SI[a]	NSI	NSI	5
4	SI	SI[a]	Neg	5
5	SI[a]	C	Neg	70
6	SI	SI[a]	SI	81
7	SI[a]	SI	SI	14
8	SI[a]	C	Neg	18
9	SI[a]	SI[a]	SI[a]	8
10	SI[a]	SI[a]	SI[a]	154
11	SI[a]	SI[a]	NSI	51
12	NSI	SI[a]	SI[a]	4
13	NSI	NSI	SI[a]	20

[a]Site of first isolation of SI phenotype.
C = contaminated culture; Neg = negative for P24 viral antigen on culture; NSI = All subsequent isolates.

RESULTS

Determination of SI Phenotype From Blood and Gut Biopsies

HIV was isolated from the blood in 45/51 patients and from the gastrointestinal tract in 35/51 patients. The SI phenotype was found in the blood of 12/45 patients. In one of these 12 patients, no concurrent biopsies were obtained. In 5/12 patients the SI phenotype was recovered exclusively from the blood (Table I).

In 8/35 patients HIV-1 SI variant was isolated from 1 or more gut tissue sites (Table I). Seven of these 8 patients had advanced HIV-1 infection (CD4 cell count < 100 cells/mm^3). The SI variant was isolated from colorectal tissue of 7/8 patients; 5 of these patients also had SI isolated simultaneously from duodenal tissue. In 1 patient, the SI phenotype was found only in the duodenal tissue. In 2 patients, we recovered the SI variant from the gut tissues while the virus in the blood had remained with the NSI-phenotype (for at least 4 months). All of these patients with SI phenotype were receiving anti-retroviral therapy at the time of biopsy.

Of 6/12 patients harboring the SI-HIV-1 phenotype in both their blood and gut, one patient showed SI-HIV-1 variant in his blood first before it appeared in the gut tissues, 3 patients developed SI variant in the blood and gut tissues simultaneously, and 2 patients showed SI-HIV-1 in the gut tissues before its appearance in the blood (Table I).

Reversion of SI-HIV-1 to NSI Variant

A reversion of the SI phenotype to the NSI variant was observed in 6 out of 12 patients harboring the SI-HIV-1 in their blood (Table II). This reversion was noted in 1 patient receiving didanosine (DDI) treat-

TABLE II. Reversion of SI Phenotype to NSI Phenotype

Patient no.	Reversion of SI to NSI in			Antiretroviral drug	CD4/mm^3 at time of reversion
	Blood	Colon	Duodenum		
1	R	S	S	AZT	19
3	R(T)	S	S	AZT	5
7	R	R	R	D4T (3TC)	16
8	R[a]	S	S	AZT	18
9	R(T)	R	R	DDI	9
11	R	R	S	AZT	91

[a]No virus recovered in subsequent visit.
R = reversed from SI to NSI; S = stable NSI; T = Transient reversion.

TABLE III. Lack of Correlation Between Plasma Viral Load and Phenotypic Switches in the Blood

Patient no.	Visit no.	Phenotype	Viral load (molecules/ml)
1	2	SI	8×10^3
	6	NSI	$<10^3$
	8	NSI	2×10^4
3	4	SI	2×10^5
	5	NSI	7×10^4
	7	SI	1×10^4
7	4	SI	5×10^4
	5	NSI	1×10^5
	7	NSI	1×10^5
8	1	SI	4×10^5
	2	NSI	3×10^5
	3	Neg[a]	3×10^5
11	1	SI	3×10^4
	2	NSI	4×10^4
	4	NSI	2×10^4

[a]Neg = negative for P24 antigen on culture.

ment, 4 patients undergoing zidovudine (AZT) treatment, and 1 patient with stavudine D4T therapy. The reversion of the SI phenotype was also noted to be transient (T) in two patients, i.e., the SI phenotype subsequently reappeared in these patients. In the other 4 patients, 2 receiving AZT treatment are still showing the NSI phenotype in their circulation after at least 4 months of their first appearance. One patient undergoing D4T therapy is harboring the NSI virus in his blood after 1 year of its first reappearance. One patient receiving AZT had no virus recovered after the appearance of the NSI by 4 months. Out of these 6 patients, 3 showed a reversion of the SI to the NSI also in their gut tissues, while the other 3 never had SI isolated from the gut sites. All of these patients were receiving a single antiretroviral drug as treatment without any modifications for at least 6 months before the switch in phenotype was noted. There was no significant difference in the CD4 positive cell counts between these patients and others who showed no fluctuation in the SI phenotype.

HIV-I Phenotype Switch and Plasma Viral Load

We investigated the relationship between the changes in the biological phenotype and the corresponding plasma viral measurement in 5/6 patients who showed a reversion in the phenotypic switches (SI to NSI). One out of 6 patients did not have corresponding plasma samples. Table III summarizes these results. One patient out of 5 had a significant drop in the RNA viremia when the switch from SI to NSI phenotype occurred. However, virus quantity did rise in subsequent visits despite the fact that the phenotype stayed as NSI. In the other 4 patients there was no significant difference in the plasma viral load when switches of phenotype occurred. Interestingly, 1 patient had no virus recovered by coculturing with PBMC (negative P24 value) in 1 visit, yet the viral load measurement was not changed from previous visits.

DISCUSSION

The SI phenotype found in the blood at advanced stages of disease could also be found in isolates from the gastrointestinal tract (Table I). This is the first documentation, to our knowledge, of the isolation of such phenotype from gastrointestinal sites. In addition, we noted that discordant phenotypes could be obtained from the blood and gut sites simultaneously. Discordant phenotypes were also noted in PBMC and lymph nodes tissues by McGavin and co-workers [1996]. It appears important, therefore, to note that the assessment of SI phenotype is not totally reflected by blood examination alone. The fact that SI-HIV-1 could be readily recovered in sicker HIV-1 patients (CD4 < 100 cells/mm^3) not only from circulating blood lymphocytes but also from 1 or more gut tissue sites suggests that HIV-1 seeds gut target site(s) such as lymphoid tissues following HIV-1 viremia, or following the inoculation of HIV-1 in gastrointestinal tract. Furthermore, the discrepancy of finding an NSI HIV-1 phenotype in the peripheral blood of some patients on anti-retroviral therapy versus recovering the SI variant from 1 or more gut tissue sites suggests that the gastrointestinal tract may be a sequestered, separate site for active viral mutation. However, it also has important implications for the strategic design of overall therapeutic approaches to combating HIV infection.

It has been proposed that the lymphoid tissues play a central part in HIV pathogenesis [Pantaleo et al., 1993]. One theory suggests that peripheral blood virus must come from, or at least represent, the virus found in the lymphoidal tissues [Coffin, 1995]. If this theory applies to the SI phenotype, then it would be unlikely that patients with SI in their blood would carry the NSI phenotype in their lymphoid tissues or vice versa, unless a switch from the SI to the NSI occurred. In fact, we observed a reversion of the SI phenotype to the NSI variant in 6/12 patients who were at the late stages of their illness as reflected by their CD4 cell count (Table II). Preliminary sequence analysis of the V3 loop done on 2 patients who showed phenotype fluctuation confirmed the switch from SI to NSI strain (data not shown). This strongly suggests that the observed switch from SI to NSI was due to some specific changes in the V3 loop.

Unlike the earlier reports [Delforge et al., 1995; Zheng et al., 1996], we noted this reversion to be occurring in patients undergoing several anti-retroviral therapies, and it was only transient in 2/6 patients (Table II). This suggests that all the anti-retroviral drugs used in this study may have a selection against the SI strain and they may indirectly select for the amino acid changes that confer for an NSI virus. Interestingly, this switch also occurred in the gastrointestinal tract in 3/6 patients, and it was not exclusive to the blood site.

The plasma viral load was unchanged in 4/5 patients when the switch took place (Table III), and in one patient there was a significant drop in the plasma viremia; however, this drop did not last and virus quantity increased to previous levels with no change in the phenotype. The CD4 cell numbers did not change during and after the switch. Unfortunately, all the patients who showed the switch from SI to NSI variants were at the late stages of the illness and demonstrated a rapid decline in clinical and biological parameters. Despite this, it is important to consider this phenomenon when examining the complex interaction among the patient's immune system, viral biological phenotype, and burden, and their effect on disease progression.

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